STUDIES ON NEW ANTIBIOTICS SF2415

II. THE STRUCTURAL ELUCIDATION

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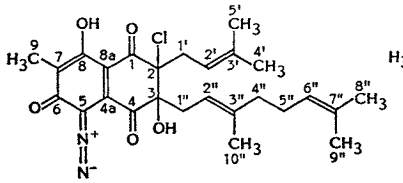
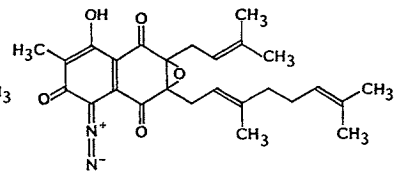
Structures of new antibiotics SF2415A1, A2, A3, B1, B2 and B3 were deduced from spectroscopic analyses and degradation studies. The structure of SF2415A3, which has the most antibacterial activity, was proposed to be 3,4a-dichloro-9-diazo-3,4,4a,10a-tetrahydro-6-hydroxy-2,2,7-trimethyl-10a-[(*E*)-3,7-dimethyl-2,6-octadienyl]-(2*H*,9*H*)naphtho-[2,3-*b*]pyran-5,8,10-trione. All of antibiotics SF2415 have a semi-naphthoquinone structure.

New antibiotics SF2415A1, A2, A3, B1, B2 and B3 (Fig. 1) which are active against Gram-positive bacteria have been isolated from the culture filtrate of *Streptomyces aculeolatus*.[1] The structures of antibiotics SF2415 were elucidated by chemical and NMR spectral studies. These antibiotics have peri-hydroxy semi-quinone structure and SF2415A1, A2 and A3 have additional unique α-diazoketone structure. In this paper we report on the structural elucidation of these antibiotics based on spectral analyses and chemical degradation studies. One of these antibiotic SF2415B3 is a methyl derivative of napyradiomycin A.[2,3]

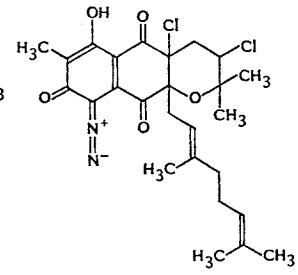
Fig. 1. The structures of SF2415.
The carbon numbers apply to the assignments of NMR.



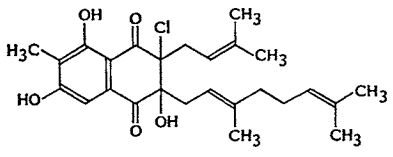
SF2415A1



SF2415A2



SF2415A3



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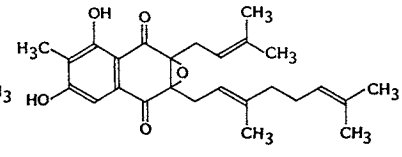

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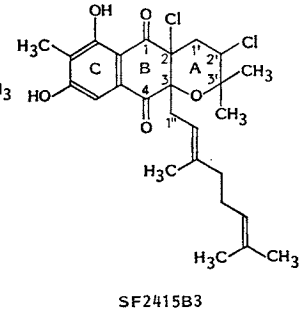


SF2415B3

The Structure of SF2415B1

The molecular formula of SF2415B1 (B1) was determined to be $C_{26}H_{33}O_5Cl$ from the elemental analysis, high-resolution (HR)-MS (M^+ *m/z* calcd 460.2015, found 460.2055), ^1H and ^{13}C NMR spectra (Tables 1 and 2). By proton selective decoupling experiments in ^1H NMR, it was clarified that B1 contained two aliphatic side chains, 3-methyl-2-butenyl group and (*E*)-3,7-dimethyl-2,6-octadienyl group. It was explained in detail that the 2′-H proton at 4.93 ppm was coupled to vicinal protons at 2.47 and 3.00 ppm (1′-H) and two allylic methyl protons at 1.58 and 1.28 ppm (4′-H and 5′-H, respectively) and these two methyl protons were coupled to homo-allylic protons (1′-H). Similarly, the 2″-H proton at 4.82 ppm was coupled to vicinal protons at 2.27 and 2.96 ppm (1″-H) and allylic methyl protons at 1.30 ppm (10″-H) and methylene at 1.89 ppm (4″-H). These methyl and methylene protons were coupled to homo-allylic protons (1″-H). And also the 6″-H proton at 5.02 ppm was coupled to vicinal protons at 1.95 ppm (5″-H) and two allylic methyl protons at 1.70 and 1.57 ppm (8″-H and 9″-H, respectively). These two methyl protons were coupled to homo-allylic protons (5″-H). To compare the C-8″ signal with C-9″ signal in ^{13}C NMR spectrum, the C-9″ signal at 17.6 ppm was observed at 8 ppm higher field than C-8″ signal at 25.6 ppm owing to steric shielding effect of the C-5″ moiety. The C-10″ signal was also observed at 16.1 ppm due to the same effect of the C-1″ moiety, therefore, the C-2″ olefin have *E* configuration. This effect was also observed between C-4′ and C-5′. The chemical shifts of the C-4″ to C-10″ carbons of this side chain are properly consistent with those of geraniol[4] and a C_{20} analogue of phytoene.[5] In ^1H NMR spectrum of B1, the remaining proton signals are one aromatic proton signal at 7.04 ppm, one aromatic methyl signal at 2.23 ppm, two phenolic proton signals at 6.66 and 12.28 ppm and one alcoholic proton signal at 4.14 ppm. IR spectrum of B1 had strong absorption bands at 1690 and 1630 cm^{-1} for semi-quinone carbonyl group and hydrogen-bonded semi-quinone carbonyl group, respectively. These semi-quinone carbonyl carbon signals were observed at 195.5 (C-1) and 196.8 (C-4) ppm in ^{13}C NMR spectrum.

The assignments of all carbon signals on ^{13}C NMR of B1 were confirmed by ^1H-^{13}C shift correlation spectral analysis (^1H-^{13}C COSY), long range ^1H-^{13}C shift correlation spectral analysis (long range ^1H-^{13}C COSY) and long range selective proton decoupling (LSPD) experiments.

The carbonyl carbon at 196.8 ppm (C-4) was coupled to the aromatic proton at 7.04 ppm (5-H) which was further coupled to the two quaternary carbons at 109.7 (C-8a) and 119.6 ppm (C-7). Enhancement of the signals at 130.7 (C-4a) and 161.4 ppm (C-6) was observed by low power irradiation at a frequency corresponding to the 5-H proton. The carbon at 161.4 ppm was coupled to the phenolic proton at 6.66 ppm. Furthermore, the aromatic methyl protons at 2.23 ppm were coupled to the three carbons at 161.4, 119.6 and 162.5 ppm (C-8). Hydrogen-bonded phenolic proton at 12.28 ppm was also coupled to the three carbons at 119.6, 162.5 and 109.7 ppm. The above-mentioned results indicated that the 5-H proton was located at the peri-position to the semi-quinone carbon at 196.8 ppm and the phenolic group, the aromatic methyl group and the hydrogen-bonded phenolic group were located at the *ortho*, *meta* and *para* position to the C-5 carbon at 106.3 ppm, respectively. Consequently, it is deduced that another semi-quinone carbon at 195.5 ppm (C-1) is hydrogen-bonded to the phenolic proton at 12.28 ppm and this was also supported by enhancement of the carbonyl carbon signal by low power irradiation of the phenolic proton at 12.28 ppm. These semi-quinone carbons C-1 and C-4 were coupled through sp^3 quaternary carbon to the 1′-H methylene protons at 2.47 and 3.00 ppm, and the 1″-H methylene protons at 2.27 and 2.96 ppm, respectively.

Table 1. ^{1}H NMR chemical shifts of SF2415.

Proton	A1	A2	A3	B1	B2	B3
	ppm m	ppm m	ppm m	ppm m	ppm m	ppm m
3-OH	3.70 br	—	—	4.14 s	—	—
5	—	—	—	7.04 s	7.17 s	7.33 s
6-OH	—	—	—	6.66 br	7.55 br	6.78 br s
8-OH	11.49 s	11.31 s	11.36 s	12.28 s	12.15 s	12.14 s
9	2.21 s	2.08 s	2.12 s	2.23 s	2.15 s	2.22 s
1′a	2.67 br dd	2.42 br dd	2.64 dd	2.47 br dd	2.41 br dd	2.43 dd
	(J=8.0, 14.5)	(J=7.0, 15.5)	(J=11.3, 14.5)	(J=8.0, 14.5)	(J=7.0, 15.5)	(J=11.3, 14.3)
1′b	2.98 br dd	3.19 br dd	2.53 dd	3.00 br dd	3.26 br dd	2.48 dd
	(J=8.0, 14.5)	(J=7.0, 15.5)	(J=4.7, 14.5)	(J=8.0, 14.5)	(J=7.0, 15.5)	(J=4.9, 14.3)
2′	4.89 br dd	5.09 br dd	4.40 dd	4.93 br dd	5.15 br dd	4.43 dd
	(J=8.0, 8.0)	(J=7.0, 7.0)	(J=4.7, 11.3)	(J=8.0, 8.0)	(J=7.0, 7.0)	(J=4.9, 11.3)
4′ or 3′-CH_3	1.59 br s	1.71 br s	1.19 s	1.58 br s	1.71 br s	1.18 s
5′ or 3′-CH_3	1.39 br s	1.72 br s	1.51 s	1.28 br s	1.73 br s	1.51 s
1″a	2.37 br dd	2.52 br dd	2.72 br dd	2.27 br dd	2.53 br dd	2.71 br d
	(J=8.0, 14.5)	(J=7.0, 15.5)	(J=7.8, 14.1)	(J=8.0, 14.5)	(J=7.0, 15.5)	(J=8.2)
1″b	3.01 br dd	3.11 br dd	2.75 br dd	2.96 br dd	3.12 br dd	2.71 br d
	(J=8.0, 14.5)	(J=7.0, 15.5)	(J=8.4, 14.1)	(J=8.0, 14.5)	(J=7.0, 15.5)	(J=8.2)
2″	4.87 br dd	5.10 br dd	4.77 br dd	4.82 br dd	5.15 br dd	4.70 br t
	(J=8.0, 8.0)	(J=7.0, 7.0)	(J=7.8, 8.4)	(J=8.0, 8.0)	(J=7.0, 7.0)	(J=8.2)
4″	1.96 m	2.01 m	1.74 br s	1.89 m	2.00 m	1.60 m
5″	2.00 m	2.06 m	1.74 br s	1.95 m	2.05 m	1.58 m
6″	5.02 br dd	5.05 br dd	4.93 m	5.02 br dd	5.05 br dd	4.87 m
	(J=8.0, 8.0)	(J=8.0, 8.0)		(J=8.0, 8.0)	(J=8.0, 8.0)	
8″	1.70 br s	1.65 br s	1.64 br s	1.70 br s	1.63 br s	1.62 br s
9″	1.59 br s	1.59 br s	1.51 br s	1.57 br s	1.56 br s	1.48 br d
						(J=0.8)
10″	1.41 br s	1.72 br s	1.38 br d	1.30 br s	1.73 br s	1.38 br d
			(J=1.2)			(J=1.2)

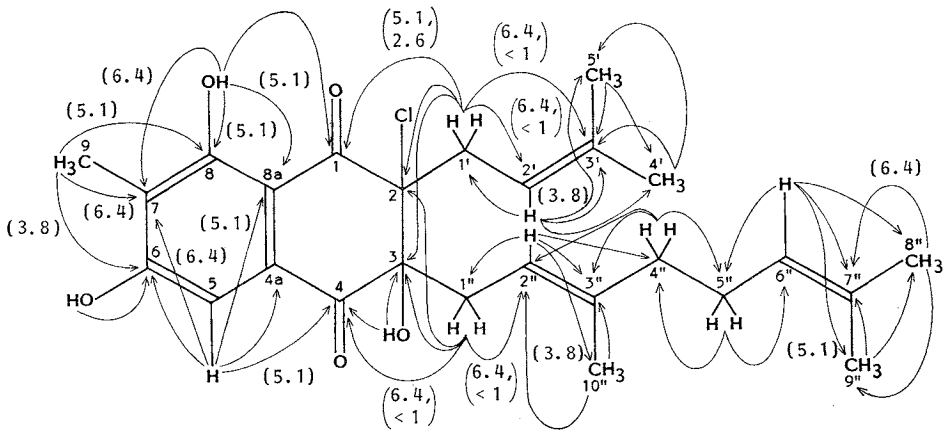
δ: ppm from TMS in $CDCl_3$. m: Multiplicity. Coupling constants (Hz) are in parentheses.

Table 2. ^{13}C NMR chemical shifts of SF2415.

Carbon	A1	A2	A3	B1	B2	B3
	ppm m	ppm m	ppm m	ppm m	ppm m	ppm m
1	193.7 s	193.7 s	192.2 s	195.5 s	192.0 s	193.4 s
2	83.0 s	66.5 s*	78.3 s	83.2 s	67.3 s*	79.1 s
3	84.2 s	67.2 s*	83.5 s	84.3 s	67.7 s*	83.4 s
4	193.1 s	188.0 s	192.9 s	196.8 s	194.9 s	196.8 s
4a	132.5 s	131.9 s	133.9 s	130.7 s	130.4 s	131.5 s
5	80.2 s	81.3 s	80.8 s	106.3 d	107.4 d	107.0 d
6	173.4 s	173.9 s	173.2 s	161.4 s	161.4 s	161.3 s
7	122.4 s	121.7 s	122.9 s	119.6 s	119.0 s	120.3 s
8	160.1 s	160.3 s	159.8 s	162.5 s	162.5 s	162.1 s
8a	113.3 s	111.0 s	112.8 s	109.7 s	108.4 s	109.4 s
9	9.3 q	9.1 q	9.3 q	8.3 q	8.3 q	8.6 q
1′	39.5 t	25.4 t	42.9 t	38.4 t	25.5 t	42.9 t
2′	116.4 d	116.1 d**	58.2 d	116.4 d	116.9 d**	58.8 d
3′	139.2 s	135.9 s	79.1 s	137.9 s	135.1 s	78.7 s
4′	26.0 q	26.0 q	22.5 q	25.7 q	26.0 q	22.4 q
5′	18.2 q	18.4 q	28.8 q	17.7 q	18.4 q	28.9 q
1″	38.5 t	25.4 t	41.7 t	37.3 t	25.7 t	41.4 t
2″	114.8 d	115.7 d**	114.4 d	115.4 d	116.6 d**	114.6 d
3″	142.5 s	139.6 s	143.2 s	141.3 s	138.5 s	142.3 s
4″	39.9 t	39.9 t	39.8 t	39.7 t	39.8 t	39.8 t
5″	26.5 t	26.6 t	26.3 t	26.3 t	26.7 t	26.1 t
6″	123.2 d	123.6 d	123.1 d	123.8 d	123.8 d	123.4 d
7″	131.9 s	131.4 s	131.7 s	131.7 s	131.2 s	131.4 s
8″	25.8 q	25.8 q	25.8 q	25.6 q	25.8 q	25.8 q
9″	17.9 q	17.9 q	17.7 q	17.6 q	17.9 q	17.7 q
10″	16.4 q	16.8 q	16.7 q	16.1 q	16.8 q	16.7 q

δ: ppm from TMS in $CDCl_3$. m: Multiplicity. *,**: Exchangeable.

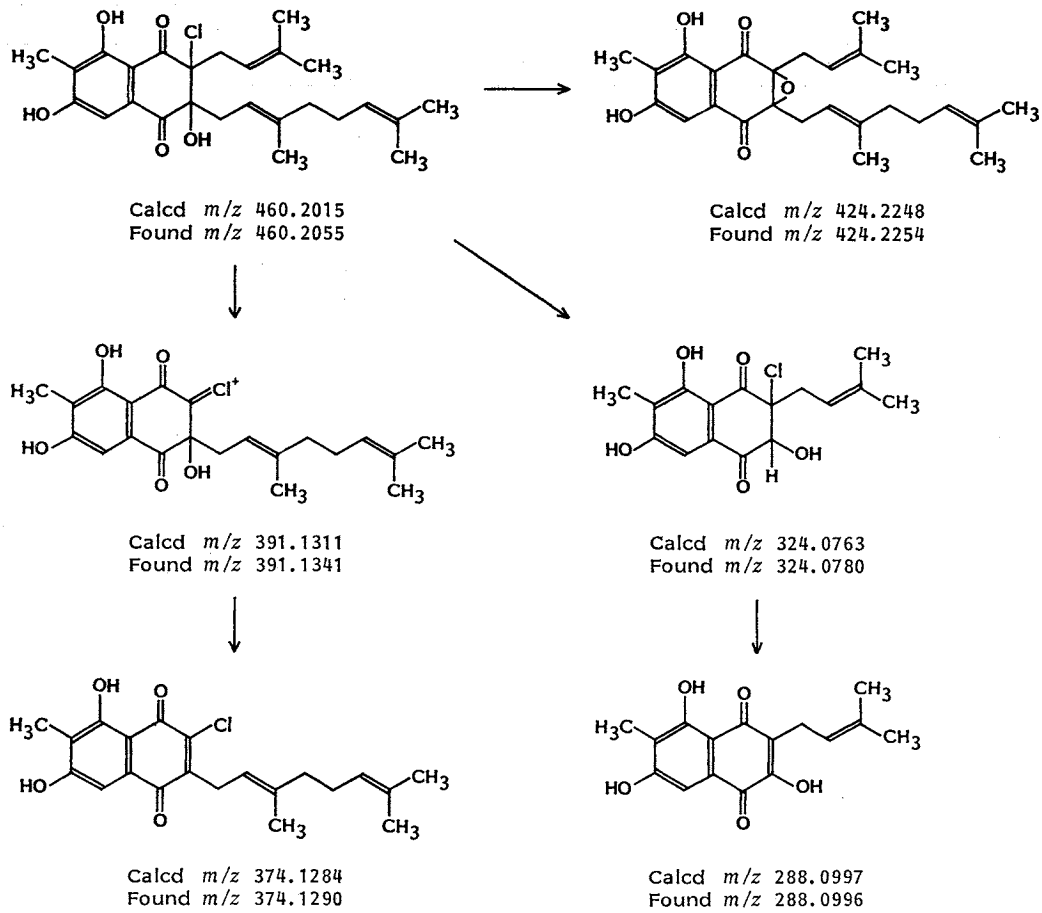
Fig. 2. The summary of LSPD experiments of SF2415B1.



Arrows indicate the carbon was coupled to the proton or enhancement of the carbon signal by irradiation of the proton, and the values in the parentheses represent the coupling constants (Hz).

Accordingly, 3-methyl-2-butenyl group and (*E*)-3,7-dimethyl-2,6-octadienyl group were attached to the C-2 carbon at 83.2 ppm and C-3 carbon at 84.3 ppm, respectively. Measurement of the proton non-decoupled ^{13}C NMR spectrum of B1 in $CDCl_3$ to which a small amount of D_2O had been added

Fig. 3. The EI mass spectral fragmentations of SF2415B1.



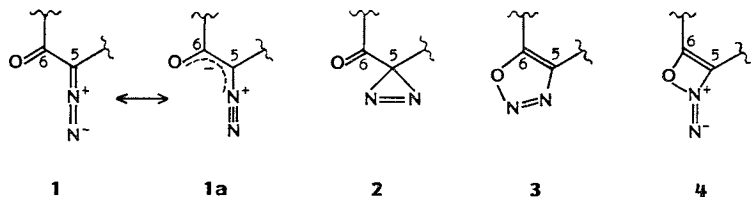
clearly showed high-field shifts of three carbon signals at 84.3, 161.4 and 162.5 ppm owing to isotope shielding effect with a marked variation of split pattern, therefore, the hydroxyl proton at 4.14 ppm connected to the C-3 carbon and this hydroxyl proton was coupled to the C-3 and C-4 carbons. Finally the remaining chlorine was connected to the C-2 carbon at 83.2 ppm which was coupled to the 1′-H and 1″-H methylene protons to which the C-3 carbon was coupled too. From these results, the structure of B1 was determined to be 2-chloro-2,3-dihydro-3,6,8-trihydroxy-7-methyl-2-[3-methyl-2-butenyl]-3-[(*E*)-3,7-dimethyl-2,6-octadienyl]-1,4-naphthalenedione.

B1 easily formed an epoxide ring by treatment with alkaline methanol resulting in the conversion to SF2415B2 (B2). This intramolecular displacement reaction may possibly proceed by an S_N2 mechanism, therefore, it may be deduced that the relative stereochemistry between the chlorine at C-2 and the hydroxyl group at C-3 was *trans*-configuration. The data of LSPD experiments were summarized in Fig. 2. Significant electron impact (EI) mass spectral fragmentations of B1 shown in Fig. 3 also supported this structure (Fig. 1).

The Structure of SF2415B2

As mentioned above, B1 ($C_{26}H_{33}O_5Cl$) was easily converted into B2 ($C_{26}H_{32}O_5$) by treatment with sodium hydroxide in aqueous methanol. This reaction was assumed that one molecule of hy-

Fig. 4. The possible structures of SF2415A1.



1 1a 2 3 4

drogen chloride was eliminated from B1 resulting in the formation of an epoxide ring. ^{1}H and ^{13}C NMR spectra of B2 revealed disappearance of hydroxyl proton signal at C-3 and high-field shifts of C-2, C-3, C-1′ and C-1″ carbon signals due to the epoxide ring formation in comparison with B1. The proton-carbon long range coupling pattern of B2 had a good resemblance to B1. From these results, the structure of B2 was determined to be 2,3-epoxy-2,3-dihydro-6,8-dihydroxy-7-methyl-2-[3-methyl-2-butenyl]-3-[(*E*)-3,7-dimethyl-2,6-octadienyl]-1,4-naphthalenedione.

The Structure of SF2415A1

When ^{1}H and ^{13}C NMR spectra of SF2415A1 (A1) were compared with those of B1, an aromatic proton signal (5-H) and a phenolic proton signal (6-OH) disappeared and the C-5 and C-6 carbon signals appeared at 80.2 and 173.4 ppm, respectively. The molecular formula of A1 was determined to be $C_{26}H_{31}N_2O_5Cl$ which was substituted two nitrogen atoms for two protons of B1 ($C_{26}H_{33}O_5Cl$). The C-6 carbon at 173.4 ppm was coupled to the aromatic methyl protons at 2.21 ppm (J=3.8 Hz) and enhancement of the C-5 carbon signal at 80.2 ppm was observed by low power irradiation of the methyl protons. As stated above, it was deduced that the difference of A1 from B1 was only at the C-5 and C-6 positions.

The possible structures at the C-5 and C-6 positions of A1 derived from the molecular formula were shown in Fig. 4. IR spectrum of A1 showed characteristic absorption band at 2155 cm^{-1} for a diazo group[6] so that **3** was precluded and **4** was also excluded because of the absence of a carbonyl group for C-6 position. The diazirine **2** is not satisfied of the IR data[7] and the signal at 173.4 ppm in ^{13}C NMR spectrum may not be assigned to the C-6 carbon. Furthermore, the UV spectrum of A1 in methanol (λ_{max} 440 nm) and *n*-hexane (λ_{max} 483 nm) revealed the presence of linear diazo structure **1**[8] conjugated semi-quinone moiety rather than **2** (Table 3). Accordingly, the structure of A1 was proposed to be 2-chloro-5-diazo-2,3-dihydro-3,8-dihydroxy-7-methyl-2-[3-methyl-2-butenyl]-3-[(*E*)-3,7-dimethyl-2,6-octadienyl]-1,4,6-(5*H*)naphthalenetrione.

The unusual chemical shifts for C-5 and C-6 in ^{13}C NMR might be explained by which A1 was of presence to be conjugate form **1a** at least in a solution (Fig. 4). The ^{13}C chemical shifts of the related diazo compounds are shown in Table 4.

A1 was slowly converted to give B1 when it was refluxed with methanol. Similarly, A1 was refluxed with methanol-d_4 to afford B1D ($C_{26}H_{32}DO_5Cl$, EI-MS *m/z* 461, 462 and 463) which was substituted by deuterium instead of the 5-H proton of B1.

The Structure of SF2415A2

Careful treatment of A1 with sodium hydroxide in aqueous methanol at room temperature gave first SF2415A2 (A2, $C_{26}H_{30}N_2O_5$) and then B2. It was deduced that the first step of this reaction was a formation of an epoxide ring and the second step was an aromatization *via* loss of nitrogen.

Table 3. UV data of SF2415.

	A1	A2	A3
λ_{max}^{MeOH} (ε)	204 (20,500),	203 (19,900),	204 (19,500),
	254 (19,200),	232 (14,400, sh),	240 (16,900, sh),
	299 (19,900),	256 (16,500),	254 (18,300),
	364 (5,500),	308 (11,900),	302 (18,900),
	440 (3,900)	371 (4,200),	376 (4,900),
		453 (2,800)	450 (3,800)
λ_{max}^{hexane} (ε)	210 (16,800),	211 (25,300),	211 (26,500),
	250 (26,200),	234 (23,000),	234 (22,500),
	281 (23,000),	250 (20,800),	248 (21,100, sh),
	295 (20,500, sh),	283 (15,400),	289 (16,400),
	373 (8,000),	307 (11,600),	300 (15,400, sh),
	483 (5,600)	380 (4,600),	377 (4,700),
		494 (3,600)	486 (3,700)

	B1	B2	B3
λ_{max}^{MeOH} (ε)	204 (20,200),	205 (23,900),	205 (21,400),
	260 (20,300),	263 (22,600),	266 (20,900),
	313 (7,800),	360 (7,400)	327 (7,800),
	347 (7,100)		360 (7,400)
λ_{max}^{hexane} (ε)	211 (25,100),	211 (25,000),	211 (23,500),
	251 (23,200),	234 (13,200),	234 (14,400, sh),
	303 (8,000),	257 (24,200),	255 (22,200),
	349 (5,300),	312 (6,200),	304 (7,400),
	360 (4,600, sh)	360 (7,400)	357 (5,600)

Table 4. ^{13}C NMR chemical shifts of diazo compounds.

Compounds	Chemical shifts
Diazomethane[10]	23.1*
DON[a]	26.7, 36.2, 54.9, 58.8*, 174.7, 198.9
SQ 30,957[b]	56.1, 69.7*, 104.0, 123.7, 126.7, 160.9, 183.3

* A carbon attached to nitrogens.

[a] 6-Diazo-5-oxo-L-norleucine.[11]

[b] 4-Diazo-3-methoxy-2,5-cyclohexadiene-1-one.[12]

In ^{13}C NMR of A2 the chemical shifts of the C-2, C-3, C-1′ and C-1″ were closely consistent with those of B2 and the chemical shifts of the C-5 and C-6 were in agreement with A1. IR spectrum of A2 indicated strong absorption band for the diazo group at 2150 cm^{-1} as well as A1. From these results, the structure of A2 was deduced to be 5-diazo-2,3-epoxy-2,3-dihydro-8-hydroxy-7-methyl-2-[3-methyl-2-butenyl]-3-[(*E*)-3,7-dimethyl-2,6-octadienyl]-1,4,6-(5*H*)naphthalenetrione.

The Structure of SF2415B3

The molecular formula of SF2415B3 (B3) was determined to be $C_{26}H_{32}O_5Cl_2$ from the elemental analysis and its spectroscopic properties. ^1H and ^{13}C NMR data of B3 are very similar to that of napyradiomycin A ($C_{25}H_{30}O_5Cl_2$) produced by *Chainia rubra* MG802-AF1.[2,3] ^1H and ^{13}C NMR spectra of B3 showed that one aromatic proton of napyradiomycin A was substituted by one methyl group in B3. It was definite by ^1H-^{13}C COSY and LSPD experiments that this methyl group was located at C-7 as well as other SF2415 antibiotics. Furthermore, nuclear Overhauser effect (NOE)

Table 5. Specific rotations of SF2415.

Solvent	A1	A2	A3	B1	B2	B3
MeOH	+133°	+49°	+195°	−82°	+122°	+33°
$CHCl_3$	−20°	+58°	+316°	−135°	+129°	+23°

All data were measured in concentration of 0.5 at 22°C using absolute MeOH and 98% pure $CHCl_3$.

difference spectrum of B3 by irradiation of 3′-methyl resonance at 1.18 ppm showed enhancement of the signals for 1′-H and another 3′-methyl group. Therefore, it was deduced that ring A adopted the chair-like conformation. The coupling constants between 2′-H proton and 1′-H protons were 11.3 and 4.9 Hz so that the chlorine atom at C-2′ was equatorial. From these data, the structure of B3 was determined to be 3,4a-dichloro-3,4,4a,10a-tetrahydro-6,8-dihydroxy-2,2,7-trimethyl-10a-[(*E*)-3,7-dimethyl-2,6-octadienyl]-(2*H*)naphtho[2,3-*b*]pyran-5,10-dione.

The Structure of SF2415A3

The ^1H and ^{13}C NMR data of SF2415A3 (A3, $C_{26}H_{30}N_2O_5Cl_2$) except for the C-5 and C-6 positions were in agreement with B3 (Tables 1 and 2) and the IR spectrum of A3 showed absorption bands for the diazo group at 2140 and 2160 cm^{-1} as well as A1 and A2 and also the chemical shifts for the C-5 (80.8 ppm) and C-6 (173.2 ppm) were consistent with those of A1 and A2. As mentioned above, the structure of A3 was deduced to be 3,4a-dichloro-9-diazo-3,4,4a,10a-tetrahydro-6-hydroxy-2,2,7-trimethyl-10a-[(*E*)-3,7-dimethyl-2,6-octadienyl]-(2*H*,9*H*)naphtho[2,3-*b*]pyran-5,8,10-trione. It supported this structure that treatment of A3 with alkaline methanol gave B3.

The data of specific rotation of SF2415 are indicated in Table 5. It is very interested that the $[\alpha]_D^{22}$ values of SF2415 antibiotics had remarkable variation induced by the difference of using solvent. Especially, although the partial configulation at C-2 to C-3 of A1 and that of B1 were the same, the signs of specific rotations in methanol were opposite to each other. This phenomenon has been particularly observed to chiral anthracyclinones.[9]

Experimental

General

^1H and ^{13}C NMR spectra were recorded on a Jeol JNM-GX400 spectrometer. The chemical shifts in $CDCl_3$ refer to an internal standard of tetramethylsilane (0 ppm). UV spectra were measured on a Shimadzu UV-260 spectrophotometer. IR spectra were recorded on a Hitachi 260-10 infrared spectrophotometer. Optical rotations were measured with a Perkin Elmer model 141 polarimeter.

Conversion of B1 into B2

To a solution of B1 (14.1 mg, 0.031 mmol) in MeOH (2.7 ml) was added 1 N NaOH (0.3 ml) at room temp. After stirring for 5 minutes, the solution was poured into water (30 ml) and acidified with 1 N HCl (0.5 ml) and extracted with $CHCl_3$ (15 ml×2). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude syrup was chromatographed on silica gel (Wakogel C-300, 2 g) with a mixture (5 : 1) of hexane and Me_2CO to give a yellow oil of B2 [11.7 mg, 90%, $[\alpha]_D^{21}$ +120° (*c* 0.5, MeOH)]. ^1H NMR, UV, IR and EI-MS spectra and Rf value on TLC of this derivative were superimposable with those of natural B2.[1]

Conversion of A1 into B1

A solution of A1 (62.0 mg, 0.127 mmol) in MeOH (5 ml) was heated at 80°C in a sealed tube for 3 hours and then evaporated to dryness. The residue was purified by preparative TLC ($CHCl_3$-MeOH, 50 : 1) to give a pale yellow oil of B1 [7.2 mg, 12%, $[\alpha]_D^{21}$ −83° (*c* 0.5, MeOH)] and recovered

A1 (48.5 mg, 78%). ^{1}H NMR, IR, UV and EI-MS spectra and Rf value on TLC of the derivative were consistent with those of natural B1.[1]

Conversion of A1 into B1D

A solution of A1 (32.6 mg, 0.067 mmol) in MeOH-d_4 (2 ml) was refluxed for 9 hours and concd to dryness. The residual oil was purified by preparative TLC (hexane - Me_2CO, 5 : 1) to afford a pale yellow oil of B1D (6.4 mg, 21%) and unchanged A1 (6.8 mg, 21%).

B1D: EI-MS *m/z* 461, 462 and 463; ^{1}H NMR ($CDCl_3$) δ 1.28 (br s, 5'-H), 1.30 (br s, 10''-H), 1.57 (br s, 9''-H), 1.59 (br s, 4'-H), 1.70 (br s, 8''-H), 1.89 (m, 4''-H), 1.95 (m, 5''-H), 2.23 (s, 9-H), 2.26 (br dd, J=14.8 and 7.8 Hz, 1''a-H), 2.47 (br dd, J=14.8 and 7.8 Hz, 1'a-H), 2.96 (br dd, J=14.8 and 7.8 Hz, 1''b-H), 3.00 (br dd, J=14.8 and 7.8 Hz, 1'b-H), 4.14 (br s, 3-OH), 4.81 (br dd, J=7.8 and 7.8 Hz, 2''-H), 4.93 (br dd, J=7.8 and 7.8 Hz, 2'-H), 5.02 (m, 6''-H), 5.88 (br, 6-OH), 12.30 (s, 8-OH).

Conversion of A1 into A2 and B2

To a solution of A1 (37.8 mg, 0.078 mmol) in MeOH (4 ml) with vigorous stirring was added dropwise 1 N NaOH (0.16 ml) at room temp for 10 minutes. The reaction mixture was poured into water (30 ml) and acidified with 1 N HCl (0.2 ml) and extracted with $CHCl_3$ (15 ml×3). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and concd to dryness. The residue was purified by preparative TLC (hexane - Me_2CO, 3 : 1) to give a red oil of A2 [7.6 mg, 22%, $[\alpha]_D^{22}$ +53° (*c* 0.5, MeOH)] and a yellow oil of B2 [9.6 mg, 29%, $[\alpha]_D^{22}$ +123° (*c* 0.5, MeOH)]. ^{1}H NMR, UV and field desorption (FD)-MS spectra and Rf values of these derivatives were in agreement with those of natural A2 and B2.[1]

Conversion of A2 into B2

A mixture of A2 (14.5 mg, 0.032 mmol), 1 N NaOH (0.3 ml) and MeOH (2.7 ml) was heated under reflux for 20 minutes. After cooling, the mixture was poured into water (30 ml) and acidified with 1 N HCl (0.5 ml) and the products were extracted with $CHCl_3$ (15 ml×3). The combined organic layers were shaken with brine, dried over anhydrous Na_2SO_4 and concd to dryness. The residual oil was chromatographed on a silica gel (Wakogel C-300, 1 g) with a mixture (5 : 1) of hexane and Me_2CO to give a yellow oil of B2 [7.4 mg, 54%, $[\alpha]_D^{22}$ +120° (*c* 0.5, MeOH)]. ^{1}H NMR, UV and EI-MS spectra and Rf value on TLC of this derivative were consistent with those of natural B2.[1]

Conversion of A3 into B3

A mixture of A3 (40.3 mg, 0.077 mmol), 1 N NaOH (0.3 ml) and MeOH (2.7 ml) was refluxed for 20 minutes. After cooling, the mixture was poured into water (30 ml) and acidified with 1 N HCl (0.5 ml) and extracted with $CHCl_3$ (20 ml×3). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and evaporated to give a crude oil. The crude oil was purified by preparative TLC with a mixture (3 : 1) of hexane and EtOAc to afford a hygroscopic yellow needles of B3 [18.7 mg, 49%, $[\alpha]_D^{27}$ +34° (*c* 0.5, MeOH)]. ^{1}H NMR, UV and FD-MS spectra and Rf value on TLC of this derivative were superimposable with those of natural B3.[1]

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